available (see figure panel B). For example, patients with inherited mutations in Jak/Stat pathway genes may benefit from ruxolitinib, patients with gain of function mutations in *STAT3* may benefit from tocilizumab, and patients with Ras-associated leukoproliferative disorder may benefit from MAPK inhibitors.[7] It is critical that we continue to study the impact of these medicines on immune health in these populations to determine whether novel targeted approaches are acting through expected mechanisms and to confirm whether they are safe for long-term use.

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RED CELLS, IRON, AND ERYTHROPOIESIS

Comment on Charlebois et al, page 271

Non–transferrin-bound iron takes the driver's seat

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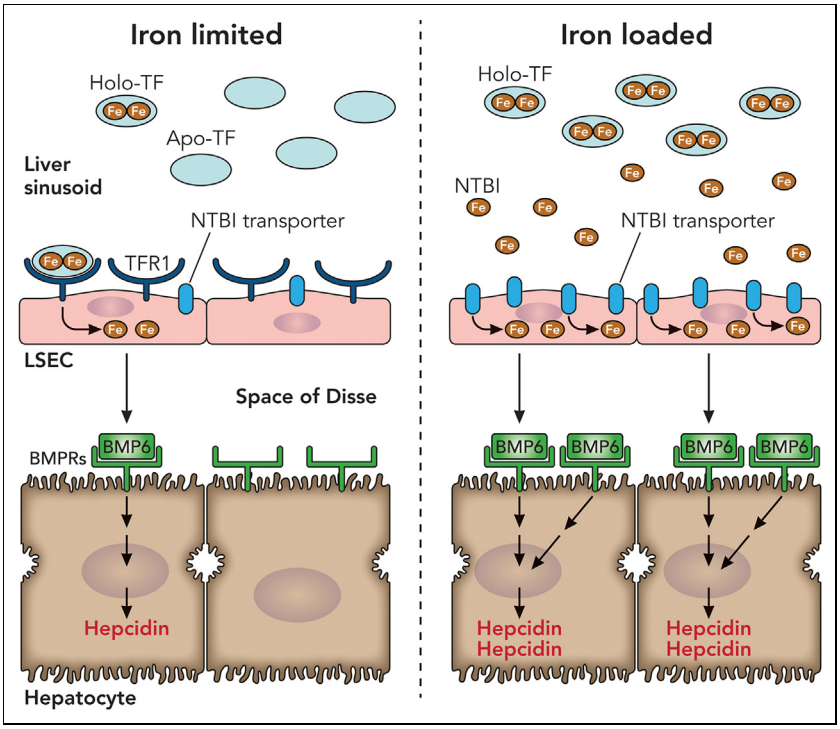
In this issue of *Blood*, Charlebois et al[1] report that non–transferrin-bound iron (NTBI) is the primary driver of bone morphogenetic protein 6 (BMP6) expression in liver sinusoidal endothelial cells (LSECs) during iron overload. This finding is important because LSEC-derived BMP6 prompts the liver to produce hepcidin, the chief iron-regulatory hormone that regulates body iron balance.

Otto von Bismarck, the "Iron Chancellor" of the German Empire in the late 19th century, argued that great questions of national policy are settled by iron and blood. One can make a similar argument for present-day research in iron biology, in which a great and unresolved question will be settled by iron. In essence, the question is how the body "senses" iron status so that it can adapt to absorb more iron when needed but avoid accumulating too much of the metal, which can be toxic in excess. Such regulation is essential for body iron balance because humans cannot excrete excess iron. An important advance in recent years has been the identification of LSECs as the site of iron sensing.[2] LSECs respond to iron loading by increasing the expression and secretion of BMP6,[3,4] which activates in neighboring hepatocytes a signaling pathway that induces the expression of hepcidin,[5] the hormone that controls how much iron the intestine absorbs. However, the form of iron taken up by LSECs that triggers *Bmp6* expression in vivo and the molecular mechanisms involved have not been well defined.

The 2 most plausible candidates for conveying the iron signal to LSECs are transferrin-bound iron and NTBI. In normal blood plasma, iron circulates nearly exclusively as transferrin-bound iron (ie, holotransferrin), which cells take up via transferrin receptor 1 (TFR1)-mediated endocytosis. In iron overload conditions, plasma iron increases to levels that exceed the iron-carrying capacity of transferrin, giving rise to NTBI, a poorly defined, heterogenous, and variable mixture that includes ferric citrate and high-mass iron aggregates.[6,7] Usually undetectable in normal healthy individuals, plasma NTBI becomes measurable when transferrin saturations surpass 70%, such as in the iron overload disorders hereditary hemochromatosis and thalassemia major. Cells take up NTBI via divalent metal-ion transporters such as ZIP14, ZIP8, and DMT1.[6] Although previous studies have shown that either holo-transferrin or NTBI (as ferric ammonium citrate) can load primary mouse liver endothelial cell cultures with iron and induce *Bmp6* expression,[3,4] how these iron sources contribute to LSEC BMP6 production in vivo requires clarification. Using mouse models and single-cell transcriptomics, Charlebois et al conclude that NTBI is the main regulator of LSEC BMP production during iron overload.

To define the role of LSEC TFR1 in the iron-dependent regulation of *Bmp6* expression, the authors generated mice with endothelial-specific inactivation of the TFR1-encoding *Tfrc* gene. They found that mice lacking endothelial TFR1 display no alterations in systemic or tissue iron levels and express normal amounts of BMP6 and hepcidin, indicating that endothelial TFR1 does not play a major



Model of iron (Fe) uptake and BMP6 production by LSECs under iron-limited and iron-loaded conditions. When the iron supply in blood plasma in the liver sinusoid is limited, LSECs become iron deficient and therefore upregulate TFR1 levels to increase iron uptake from holo-TF. Conversely, iron loading of LSECs downregulates TFR1 levels, thus limiting iron uptake via holo-TF. When iron levels exceed TF's iron-carrying capacity, NTBI appears in the plasma and is taken up by LSECs via a plasma membrane NTBI transporter, which remains to be identified. In iron overload, NTBI becomes the main driver of LSEC production of BMP6, which binds to BMP receptors (BMPRs) on neighboring hepatocytes, thereby activating a signaling pathway that increases the liver's synthesis of hepcidin. Under iron-limited conditions, LSECs acquire iron via holo-TF and TFR1, which is needed for appropriate basal production of BMP6 and hepcidin. Professional illustration by Patrick Lane, ScEYEnce Studios.

role in iron homeostasis. However, when fed an iron-deficient diet, mice lacking endothelial TFR1 displayed reduced expression of hepatic BMP6 and hepcidin relative to liver iron content, suggesting a minor requirement for endothelial TFR1 during iron deficiency. The need for TFR1 under these conditions makes sense because cells respond to iron deficiency by upregulating TFR1 levels to acquire more iron from holo-transferrin. Of note, a nearly identical iron phenotype and iron-deficiency response was recently reported by Fisher et al,[3] who studied mice lacking endothelial TFR1 generated by using a different Cre mouse line (ie, *Stab2*-Cre vs *Tek*-Cre).

In their study, Charlebois et al additionally demonstrated that feeding mice a high-iron diet, which elevated transferrin saturations to more than 90%, increased hepatic expression of *Bmp6* and hepcidin similarly between wild-type mice and those lacking endothelial TFR1. Moreover, plasma NTBI concentrations of mice of either genotype correlated positively and significantly with hepatic *Bmp6* and hepcidin messenger RNA (mRNA) levels. Single-cell transcriptomic analyses of liver cell types from wild-type mice further revealed a more robust induction of *Bmp6* mRNA in LSECs following dietary iron loading (and NTBI formation) than from a single intravenous bolus of exogenous holo-transferrin. Together, these data imply that LSEC uptake of NTBI, rather than transferrin-bound iron via TFR1, drives *Bmp6* and hepcidin expression in response to iron loading (see figure). Future studies need to define temporal and dose-response effects of plasma NTBI (eg, exogenously administered NTBI) on *Bmp6* induction and hepcidin expression in vivo.

But how do LSECs take up NTBI? The most well-characterized NTBI transporter, ZIP14 (SLC39A14), which mediates NTBI uptake by hepatocytes, appears dispensable as previous studies showed that *Slc39a14* knockout mice efficiently upregulate hepatic *Bmp6* and hepcidin expression in response to short- or long-term dietary iron loading or genetic iron overload.[8] Seemingly consistent with this idea, the authors' single-cell transcriptomic analyses of LSECs from wild-type mice found that *Slc39a14* mRNA levels did not increase in response to a high-iron diet. The transcriptomic analyses did reveal, however, that the high-iron diet strongly induced the expression of *Slc39a8* encoding ZIP8, which like ZIP14, can transport NTBI, at least in cultured cells.[9] This finding, along with the observation that LSECs abundantly express *Slc39a8*,[10] identifies ZIP8 as a lead candidate for NTBI uptake by LSECs.

If iron acquired from NTBI per se proves to be the dominant signal for BMP6 induction by LSECs in vivo, follow-up studies will need to identify which chemical species of NTBI mediates the effect. This new knowledge, combined with identifying the LSEC NTBI transporter(s), not only will add to the repertoire of possible therapeutic targets for treating iron overload disorders but will also help to fill the gap in our understanding of how iron regulates its own homeostasis.

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THROMBOSIS AND HEMOSTASIS

Comment on Doyle et al, page 285

iTTP: more long-term consequences

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Immune thrombocytopenic purpura (iTTP) is a near-fatal disease unless immediate treatment is initiated with the current recommended therapies of therapeutic plasma exchange, immune suppression, and caplacizumab.[1,2] With this regimen, the mortality of iTTP in high-volume centers has been markedly reduced, transforming it instead into a chronic disease, where relapse and recurrent episodes of thrombotic microangiopathy (TMA) can occur. However, aside from ADAMTS13 (a disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13) deficiency, factors that increase the risk for relapse are not well understood. To address these questions, in this issue of *Blood*, Doyle et al used a large and well-established national cohort of patients with iTTP in the United Kingdom, reviewing the incidence and potential risk factors of relapse in patients with iTTP, with special attention paid to the use of anti-CD20 therapies.[3]

The authors identified 443 patients with at least 3 years of follow-up (specifically, a median follow-up of 8.6 years). Of these patients, 30% had at least 1 relapse during the study period, which was defined as either a decline in ADAMTS13 activity to <20% (ie, "ADAMTS13 relapse") or a "clinical relapse," which was a recurrence of TMA. Given the follow-up that was available, a relapse rate of 4% within the first year, reaching 40% within 5 years, was observed. The authors compared ADAMTS13 with clinical TMA relapses before and after 2012, noting that clinical relapses decreased from 23% to 11%, whereas ADAMTS13 relapses increased from 8% to 16%.

More than 50% of patients received anti-CD20 therapies. with most receiving rituximab. Remission of ADAMTS13 inhibition, defined as reaching an activity level of >20%, occurred in a median time of 21 days, with peak activity seen at 3 months. For those patients with iTTP who were followed up to 10 years, no difference was seen between those who had or had not received anti-CD20 therapy with time to first relapse, as immune reconstitution leads to relapse. As in earlier reports on preemptive therapy, Doyle et al observed that in those patients with iTTP who responded to anti-CD20 therapy initially, the patients did so again at time of relapse on retreatment with anti-CD20 therapy. And although standard initial therapies were effective for most patients with iTTP, Doyle et al observed that 28 of 443 (6%) had "frequent" relapses, defined as ≥0.5/year, requiring frequent retreatment.

As to risk factors for relapse, patients with iTTP with a "reversible" cause, such as medication or infection induced, had a lower rate of relapse vs those who did not (8% vs 16%). Most important, the authors also observed that Black-Caribbean race (ie, African descent) was associated with a higher risk of relapse (17% vs 7%), a finding that mirrors those initially reported in *Blood*.[4]

Many of these findings are known to experts within the field of thrombocytopenic purpura and reaffirm current practices. However, as would be expected with the retrospective nature of this study, significant changes occurred over the course of this study in the diagnosis, treatment, and management of iTTP. As a result, some of these findings are limited and will require prospective confirmation.

So, what does this study tell us? The findings reported by Doyle et al support the following observations: (1) an "immunologic" relapse can occur in patients with iTTP years after achieving remission, leading to ADAMTS13 deficiency; (2) ADAMTS13 deficiency leads to recurrent episodes of TMA; (3) regular surveillance and "preemptive" rituximab can raise the ADAMTS13 activity level and prevent TMA; and (4) patients with iTTP of African descent had an increased risk of relapse. Again, these observations support the assertion that iTTP should be considered a long-term disease, with a significant proportion at risk for relapse. This long-term risk of relapse in iTTP, be it an ADAMTS13 or clinical relapse, is also associated with higher rates of depression, cardiovascular disease, neurocognitive decline, and posttraumatic stress disorder.[5-7]

As with any good study, more questions requiring study have been raised. It is still unclear what factor(s) cause the emergence, let alone the recurrence, of the ADAMTS13 antibody. Although the current treatment of plasma exchange, immune suppression with anti-CD20 therapy, and caplacizumab is effective in reducing the risks of exacerbation, relapse, thrombosis, and death, the best treatment for ADAMTS13 inhibitor eradication remains unknown for those patients who do not respond to anti-CD20 treatment. Likewise, recommendations for ADAMTS13 testing are unclear, with International Society on Thrombosis and Haemostasis guidelines offering no explicit time frame,[1] but the US Thrombotic Microangiopathy Alliance recommending testing every 3 months